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Development of low cost medium for the culture of *Chlorella ellipsoidea* using poultry waste

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Abstract: *Chlorella ellipsoidea* was cultured in different concentrations viz., 30, 40, 50, 60 and 70% of poultry waste medium (PWM). Bold basal medium (BBM) was used as control. Among the different concentrations, 50% PWM showed significantly higher ($p<0.05$) growth in respect to cell number, chlorophyll a, specific growth rate of cell number, chlorophyll a and total biomass than other concentrations except control. The physico-chemical parameters of the culture media were found within the suitable range of microalgae culture. Proximate composition analysis showed the cultured algae was rich in protein, lipid and other mineral contents. Protein percentage of cultured *C. ellipsoidea* was significantly higher ($p<0.05$) in 50% PWM concentration than other concentrations of PWM.

Keywords: *Chlorella ellipsoidea*, poultry waste, low cost medium

Introduction

Chlorella ellipsoidea is a very common microalgae in Bangladesh as well as all over the world. It is a fast growing microalgae rich in different kinds of nutrients (Habib, 1998, Becker, 1978). *Chlorella* is one of the nutritious foods in nature and one of the simplest, primitive cells, yet science may never be able to unravel all its mysteries (Jensen, 1989). The entire cell of *Chlorella* contains all the nutrients necessary to sustain life (Kane, 1989). Using inorganic media, laboratory base microalgae culture in Bangladesh is very costly due to unavailability of different ingredients of media. So it is essential to find out inexpensive culture media using available ingredients. Poultry waste is available in Bangladesh. In Bangladesh near about 1 lac poultry farms are discharging huge amount of waste in nature every year, which pollute aquatic environment partially and increases both biological oxygen demand (BOD) and chemical oxygen demand (COD) in aquatic environment. So, the present experiment was under taken to culture *C. ellipsoidea* using different concentration of poultrty waste and to find out the nutritional values of the cultured microalgae.

Materials and Methods

For the culture of *Chlorella ellipsoidea* five different concentrations (treatment) of 20 days aerated poultry waste media (PWM) i.e. 30, 40, 50, 60 and 70% of 100% PWM and as control Bold basal medium (BBM) were used in 1L conical flask with three replications of each concentration. The cell number, chlorophyll a content, optical density, temperature, light intensity, dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and posphate-phosphorus (PO_4-P) of the culture media were analyzed. Besides, specific growth rate (SGR μ/day) in respect to cell and chlorophyll a content and total biomass on the basis of chlorophyll a were determined following the methods of Clesceri *et al*. (1989). Proximate composition of *C. ellipsoidea* cultured in different concentrations of PWM was analysed to determine the nutritional values following Horwitz (1984). To compare the treatment means for the parameters recorded, one way ANOVA was performed using SPSS package followed by DMRT (Zar, 1984).

Results

The highest ($p< 0.05$) cell number of *Chlorella ellipsoidea* 198.49×10^5 cells /ml was recorded in control (BBM) followed by 50, 60, 40, 70 and 30 % PWM (Table 1) on the 10^{th} day of the culture. Similar trend was observed in case of chlorophyll-a content and optical density of *C. ellipsoidea*. The range of Chlorophyll a content was 5.85 to 9.39 mg/l and optical density at 620 nm was 0.98 to 1.95 (Table 1). Highest pH of the media was recorded on the stationary phase, which ranged from 7.94 to 8.42 for all the treatments (Table 1). An increasing trend of dissolved oxygen (DO) was recorded up to the stationary phase, after that it was decreasing and the highest DO was recorded 5.17 to 5.47 mg/l on the 10^{th} day of culture among the all treatments (Table 1). The range of light intensity and temperature were 2170 to 2205 lux/m^2/s and 28.0 to 29.90^oC, respectively during the whole culture period of the present study. The specific growth rate (SGR, μ/day) on the basis of cell number and chlorophyll a content were found in the range of 0.40 to 0.44 and 0.41 to 0.45, respectively when *C. ellipsoidea* grown in different concentrations of PWM and BBM (Table 16). Total biomass of *C. ellipsoidea* in respect to chlorophyll-a content was found significantly ($p<0.05$) higher when grown in BBM than cultured in 50% PWM followed by 60, 40, 70 and 30% PWM. But total biomass in 50% PWM was significantly ($p<0.05$) higher than that of grown in other concentrations of PWM (Table 2)

During the culture period a decreasing trend of both PO_4-P and NO_3-N was observed for all the treatments up to stationary phase and next it was increasing. At the stationary phase the content of PO_4-P was recorded 1.59, 2.65, 2.98, 3.45, 4.45 and 4.99 mg/l in 30, 40, 50, 60, 70% PWM and BBM respectively (Table 1). Significantly ($p<0.05$) higher amount of NO_3-N was recorded in 50 and 70% PWM followed by 60, 40, 30% PWM and BBM at the stationary phase (Table 1).

Table 1: Growth performances of *Chlorella ellipsoidea* grown in different concentrations of PWM and BBM

Day	30% PWM	40% PWM	50% PWM	60% PWM	70% PWM	BBM
a. Mean (± SD) cell weight						
8	103.86±5.21	130.63±8.27	146.27±6.46	136.70±7.27	124.35±6.18	155.52±7.48
10	121.37±9.50	142.34±9.46	182.07±9.02	157.41±12.06	137.57±16.80	198.49±22.07
12	112.39±7.58	131.77±7.35	169.30±9.24	147.55±9.61	127.37±14.23	185.33±14.32
b. Mean (± SD) Chlorophyll a content (mg/l)						
8	4.24±0.16	5.75±0.24	6.46±0.16	5.65±0.25	4.91±0.13	6.29±0.17
10	5.85±0.12	5.75±0.24	8.08±0.13	7.06±0.21	6.55±0.30	9.39±0.30
12	4.71±0.24	5.75±0.24	7.60±0.16	6.74±0.27	5.74±0.19	8.96±0.25
c. Mean (± SD) Optical density at 620 nm						
8	0.80±0.02	1.00±0.03	1.22±0.02	1.11±0.00	0.94±0.02	1.41±0.03
10	0.98±0.03	1.18±0.04	1.67±0.04	1.42±0.02	1.11±0.03	1.95±0.03
12	0.82±0.02	1.06±0.01	1.47±0.01	1.21±0.00	0.97±0.01	1.88±0.04
d. Mean (± SD) pH						
8	7.63±0.05	7.76±0.03	7.78±0.02	7.81±0.05	7.85±0.03	7.86±0.07
10	7.94±0.04	8.01±0.02	8.24±0.04	8.18±0.07	8.07±0.07	8.42±0.05
12	7.73±0.06	7.89±0.05	7.92±0.09	7.87±0.06	7.83±0.05	8.23±0.04
e. Mean (± SD) Dissolved oxygen (mg/l)						
8	4.27±0.06	4.43±0.07	4.60±0.04	4.53±0.07	4.23±0.07	4.73±0.06
10	4.37±0.07	4.60±0.08	4.83±0.06	4.73±0.08	4.33±0.06	5.03±0.05
12	4.20±0.04	4.40±0.02	4.64±0.08	4.33±0.08	4.03±0.07	4.63±0.08
f. Mean (± SD) PO_4-P (mg/l)						
8	2.13±0.06	3.03±0.05	3.33±0.08	4.16±0.05	5.06±0.05	5.35±0.05
10	1.59±0.06	2.65±0.04	2.98±0.06	3.45±0.04	4.45±0.06	4.99±0.05
12	1.92±0.05	2.96±0.06	3.42±0.05	3.84±0.07	4.85±0.08	5.36±0.07
g. Mean (± SD) NO_3-N (mg/l)						
8	9.47±0.13	10.50±0.11	11.83±0.14	11.83±0.11	12.17±0.11	8.10±0.011
10	8.57±0.14	9.60±0.12	11.10±0.11	10.47±0.11	11.23±0.13	7.27±0.09
12	8.80±0.07	9.87±0.14	11.33±0.08	10.87±0.09	11.07±0.12	7.57±0.11
h. Mean (± SD) NO_2-N (mg/l)						
8	9.83±0.21	10.14±0.27	10.46±0.13	10.63±0.17	11.17±0.20	0.16±0.03
10	10.15±0.25	10.61±0.18	10.63±0.24	10.66±0.24	11.63±0.27	0.17±0.04
12	10.86±0.24	10.59±0.12	11.21±0.27	11.46±0.27	12.32±0.24	0.42±0.02
i. Mean (± SD) NH_3-N (mg/l)						
8	0.85±0.03	1.02±0.05	1.01±0.06	1.15±0.05	1.26±0.06	0.26±0.03
10	1.01±0.04	1.15±0.03	1.08±0.05	1.32±0.07	1.44±0.04	0.39±0.03
12	1.51±0.05	1.58±0.08	1.66±0.07	1.69±0.04	1.93±0.05	0.69±0.04
j. Mean (± SD) temperature (^{O}C)						
8	29.10±0.01	29.17±0.01	29.17±0.03	29.23±0.04	29.20±0.07	29.27±0.01
10	28.73±0.04	28.67±0.02	28.80±0.04	28.73±0.05	28.70±0.05	28.67±0.05
12	29.53±0.02	29.53±0.04	29.47±0.01	29.63±0.03	29.504±0.06	29.90±0.03
k. Mean (± SD) light intensity (lux/m^2/s)						
8	2196.37±7.32	2194.28±7.34	2189.29±8.24	2183.33±6.14	2189.12±2.65	2184.26± 8.18
10	2196.37±7.32	2205.61±6.27	2184.34±9.17	2183.26±7.35	2194.42±4.38	2188.15±9.47
12	2176.28±7.59	2175.47±9.24	2171.27±5.39	2170.72±6.28	2174.37±7.28	2176.47±8.31

On the other hand NO_2-N showed an increasing trend with the age of the culture. At the stationary phase NO_2-N was found 0.15, 10.61, 10.63, 10.66 and 11.63 mg/l in 30, 40, 50, 60, and 70% PWM, respectively and 0.17 mg/l in BBM (Table 1). The data showed that very less amount of NO_2-N was recorded in BBM than that of all concentrations of PWM. The content of NH_3-N gave the similar trend like NO_2-N and the range was 0.39 to 1.44 mg/l at the stationary phase (Table 9). During the study period the range of temperature and light intensity was recorded 28.00 to 29.90°C and 2170 to 2205 lux/m^2/s respectively (Table 1).

The proximate composition of *C. ellipsoidea* cultured in different concentrations of PWM as well as BBM was analyzed. The crude protein crude lipid, ash, crude fiber, moisture and NFE (%) were recorded which ranged from 33.26 to 44.63, 7.63 to 8.66, 10.49 to 13.14, 4.65 to 7.95, 9.73 to 10.25 and 17.35 to 26.64 respectively, for all the treatments including BBM (Table 3).

Table 2. Mean (± SD) specific growth rate (μ/day) of cell, chlorophyll a (chl-a) and total biomass (mg/l) of *Chlorella ellipsoidea* grown in different concentrations PWM and BBM

Parameters	30% PWM	40% PWM	50% PWM	60% PWM	70% PWM	BBM
SGR of cell	0.40 ± 0.00^{e}	0.40 ± 0.00^{d}	0.43 ± 0.01^{b}	0.41 ± 0.00^{c}	0.40 ± 0.01^{de}	0.44 ± 0.00^{a}
SGR of chlo-a	0.41 ± 0.01^{d}	0.42 ± 0.01^{cd}	0.44 ± 0.01^{b}	0.43 ± 0.00^{c}	0.42 ± 0.01^{cd}	0.45 ± 0.00^{a}
Total biomass (Chlo-a x 67)	392.17 ± 29.18^{d}	463.86 ± 19.92^{c}	541.36 ± 3.55^{b}	473.69 ± 14.42^{c}	438.85 ± 21.44^{e}	629.13 ± 20.77^{a}

Different superscripts in each row indicate significant differences ($p < 0.05$)

Table 3. Mean values (±SD) of proximate composition (%) of *Chlorella ellipsoidea* cultured in different concentrations of PWM and BBM

Composition	30% PWM	40% PWM	50% PWM	60% PWM	70% PWM	BBM
Crude Protein	4.83 ± 0.06^{c}	37.27 ± 0.08^{b}	4.63 ± 0.11^{a}	34.86 ± 0.04^{c}	33.26 ± 0.11^{d}	43.47 ± 0.11^{a}
Crude Lipid	$.80 \pm 0.06^{b}$	0.65 ± 0.10^{b}	11.66 ± 0.06^{a}	11.56 ± 0.16^{a}	10.63 ± 0.19^{b}	10.85 ± 0.29^{b}
Ash	3.14 ± 0.12^{a}	10.71 ± 0.29^{b}	10.49 ± 0.53^{b}	13.06 ± 0.18^{a}	12.32 ± 0.14^{a}	10.51 ± 0.21^{b}
Crude Fiber	4.77 ± 0.05^{cd}	4.65 ± 0.06^{d}	5.26 ± 0.03^{b}	5.13 ± 0.09^{b}	4.90 ± 0.02^{c}	7.95 ± 0.03^{a}
Moisture	9.99 ± 0.12^{b}	10.08 ± 0.19^{ab}	9.73 ± 0.11^{c}	9.85 ± 0.09^{bc}	10.25 ± 0.06^{a}	9.94 ± 0.15^{bc}
NFE	26.47 ± 0.26^{b}	26.64 ± 0.13^{b}	18.23 ± 0.28^{d}	25.54 ± 0.23^{c}	28.64 ± 0.37^{a}	17.35 ± 0.24^{d}

Different superscripts in each row indicates significant differences ($p<0.05$)

Discussion

In this study different concentrations of PWM were used with control as BBM. It was found that highest cell number 198.49×10^5/ml was recorded on the 10^{th} day of the culture in BBM followed by 50, 60, 40, 70 and 30% PWM. Among the different concentrations of PWM the cell number grown in 50% PWM (182.07×10^5/ml) was significantly ($p<0.05$) higher than grown in other concentrations of PWM. It might be happened due to the presence of sufficient amount of nutrient in 50% PWM. Same species of microalgae was cultured by Alam *et al.* (2000) in different inorganic media. He found that *Chlorella ellipsoidea* attained a maximum cell density of 282.77×10^5/ml in modified Nichols medium (MNM) followed by 215.00×10^5 cells/ ml in BBM and 159.42×10^5/ml in NPK medium on the10^{th} day of culture. It was observed that the growth of 50% PWM and BBM of the present work were within the range of the findings of Alam *et al.* (2003). Habib (1998) worked on *Chlorella vulgaris* in rubber effluent media and recorded the highest cell no. of *C. vulgaris* 285.20 x 10^5/ml on the 10^{th} of the culture which was higher than the present findings. These variations might be due to the difference of media as well as the media concentrations. Chlorophyll a content and optical density was also increasing up to the stationary phase and after that these were decreasing. The direct relation of the cell number, chlorophyll a content and optical density proves that the culture was justified which happened due to the proper management of the culture. The findings of Karmaker *et al.* (2001) regarding chlorophyll a content and optical density was lower than that of the present study. On the other hand Habib (1998) and Islam *et al.* (2004) recorded higher chlorophyll a content and optical density than the present study. These variations might be due to the variations of media and difference of cell density grown in the media. In the present experiment, pH gave an increasing trend up to the stationary phase and highest pH observed 8.42 on the 10^{th} day of the culture. Karmaker *et al.* (2001) recorded the highest pH 7.83 in his study of *C. ellipsoidea* in ripe bean seed powder (RBSP) media. Dissolved oxygen was ranged from 4.37 to 5.09 during maximum cell growth of *C. ellipsoidea*. Karmakar *et al.* (2001) observed maximum DO 5.75 and 4.49 mg/l respectively when cultured *Chlorella sp*. in different organic media. Light intensity was ranged 2170.72 to 2205.61 $lux/m^2/s$ during the culture period in the present study. Karmakar *et al.* (2001) used light intensity 2410 to 2490 $lux/m^2/s$ when cultured *Chlorella ellipsoidea*. The SGR of *C. ellipsoidea* on the basis of cell was in the range of 0.40 to 0.44 and on the basis of chlorophyll a content the range was recorded 0.41 to 0.45. The SGR of *C. ellipsoidea* on the basis of cell was significantly ($p<0.05$) higher in BBM than other treatments. In respect to chlorophyll a the highest ($p<0.05$) total biomass was 629.13 mg/l in BBM followed by 541.36, 473.69, 463.86, 438.85 and 392.17 mg/l in 50, 60, 40, 70 and 30% PWM respectively. The SGR and total biomass in 50% PWM was significantly ($p<0.05$) higher than other concentrations of PWM (Table 2). On the basis of chlorophyll a content the SGR were recorded 0.30 to 0.42 and 0.30 to 0.40, respectively and the total biomass was found 438.85 to 812.04 mg/l. These findings are more or less similar to the present findings. Both the contents of NO_3-N and PO_4-P were higher on the first day of culture in different concentrations of PWM and BBM. The values were decreasing up to the stationary phase for all the treatments, which might be happened due to the utilization of NO_3-N and PO_4-P by cells. At the stationary phase highest amount of NO_3-N (11.26 mg/l) was recorded in 60% PWM which was significantly ($p<0.05$) higher than all other treatments. At the stationary phase the content of PO_4-P recorded in BBM (4.99 mg/l) was significantly ($p<0.05$) higher than recorded in 30, 40, 50, and 60% PWM. Here it is clear that PWM contained higher amount of NO_3-N and less amount of PO_4-P than control media, BBM. The increasing trend of NO_3-N and PO_4-P after the stationary phase might be happened due to the presence of decomposed dead cells. The NO_2-N and NH_3-N showed an increasing trend with the age of culture in different concentrations of PWM and BBM. Significantly ($p<0.05$) higher amount of NO_2-N and NH_3-N were recorded in all concentrations of PWM than BBM at the stationary phase. Proximate composition analysis showed that significantly ($p<0.05$) higher percentage of protein was recorded, when *C. ellipsoidea* cultured in 50% PWM than other treatments except BBM. The percentage of crude lipid of *C. ellipsoidea* grown in 50% PWM was significantly higher than that of grown in other treatments including BBM. The ash content of *C. ellipsoidea* grown in 50% PWM and BBM varied insignificantly ($p<0.05$). The present findings somehow agree with the findings of Becker (1978), Soeder (1980), and Habib (1998). Considering the proximate composition of the microalgae it may be concluded that *C. ellipsoidea* was found nutritionally rich when cultured in different concentrations of PWM and 50% PWM showed the best performance.

Conclusion

Culture of *C. ellipsoidea* might be established in Bangladesh using the inexpensive poultry waste as media, which ultimately promote culture based aquaculture system and environment will be partially free from pollution.

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